

The University of Chicago

METAL CATALYSTS IN BIOLOGIC OXIDATIONS

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
PHYSICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

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By
ROMEO RALPH LEGAULT

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METAL CATALYSTS IN BIOLOGIC OXIDATIONS*

THE SIMPLE SYSTEM: THIOGLYCOLIC ACID, BUFFER, METAL, DITHIOL

By R. R. LEGAULT

It is well recognized that heavy metal ions play a major catalytic rôle in controlling the course and speed of metabolic reactions, especially oxidations, of various substrates and intermediates, in cells or *in vitro*. The metal ions in the cell, however, are rarely active as such, but form a wide array of complexes, of which some are highly catalytic, others quite inert. For reasons developed more completely in Paper II, I considered it not improbable that the normal balance of cell oxidation might be achieved by the presence in cells of two sorts of substances competing with each other for the metals and forming, respectively, active and inactive complexes. Such augmentor and especially inhibitor substances could easily be demonstrated in tissue extracts if a quantitative method of measuring the catalytic activity of metal ions were available, since the extracts could be added to a standardized system and the change in activity noted.

The pioneer contribution of Warburg (1927), who estimated metal ions in tissue by the effectiveness of its ash in catalyzing cysteine oxidation, has been further exploited by many competent workers, and I had hoped to find such a method applicable to my problem. When, however, the more complex situation met in tissue extracts, as compared to ash, was encountered, it was

* This work was made possible by assistance from the Elizabeth Storck Kraemer Memorial Research Fund, founded by Mr. Pierre S. du Pont.

soon apparent that a more exhaustive study of the method itself was a necessary preliminary undertaking. When this analysis was largely completed, several careful studies appeared, which my own findings in part confirm; in part, extend.

The present paper reports an exploration of the catalytic action of iron, copper, and manganese ions on the oxidation of thioglycolic

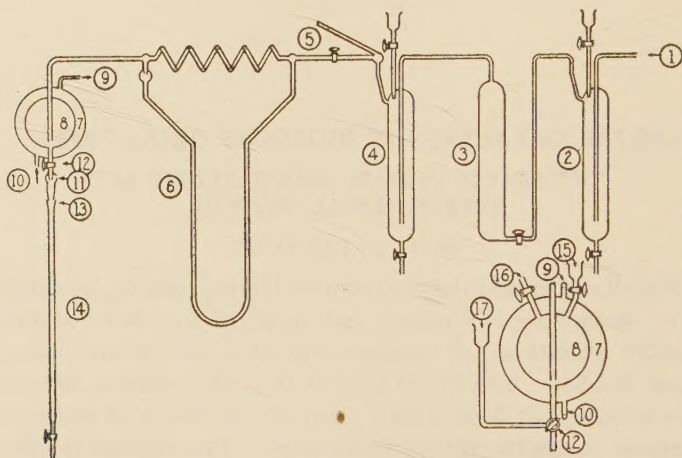


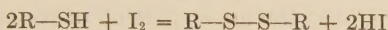
FIG. 1. Apparatus for the titrimetric method of measuring rates of oxidations. 1, oxygen intake; 2, alkaline permanganate wash vessel; 3, finely divided silica gel, held in place by cotton plugs to remove suspended particles; 4, water wash bottle to resaturate gas; 5, fine stop-cock to adjust gas flow; 6, flowmeter containing a good grade of mineral oil and a dye; 7, outer water jacket to maintain temperature constant (10 and 9, intake and return to thermostat); 8, reaction vessel; 11, aperture to allow escape of air while burette is filled; 12, 3-way stop-cock; 13, ground joint to burette; 14, 25 cc. burette; 15, funnel for introduction of materials into reaction vessel; 16, oxygen exit during reaction; 17, arm that connects through 3-way stop-cock (12) with burette (14) and permits washing of latter between titrations.

acid as the following factors are varied: temperature, oxygen tension, initial thiol concentration, initial dithiol concentration, hydrogen ion concentration, character of buffer, especially phosphate and carbonate, combinations of ions, specific ion inhibitors—cyanide, pyrophosphate, and the like. Thioglycolic acid was employed in my studies because it is easily prepared, gives repro-

ducible results, and may be considered as fairly analogous to substances actually participating in important cell oxidation reactions.

Methods

It is apparent that the progress of the oxidation of thioglycolic acid may be followed by measuring the volume of oxygen consumed or the amount of unoxidized thiol remaining. For the former determination, the manometric method is quite satisfactory; for the latter, periodic titrations with a standard iodine solution are required. The reaction involved is



The titrimetric method is more time-consuming than the manometric, since fewer experiments may be performed simultaneously, but it might have proved more specific in the presence of complex substances, as proteins, derived from tissue. I have, therefore, used both methods, though, as it developed, both gave closely concordant results for the systems studied.

Apparatus—For the titrimetric measurements, the oxidation was effected in the apparatus shown in Fig. 1. Manometric measurements were carried out by the method of Warburg (1927), as modified by Gerard (1931).

Reagents—Doubly distilled water was used in the preparation of all solutions, as well as in the crystallization of solid reagents. Liquids were also distilled twice before use. All reagents were stored in Pyrex bottles provided with ground glass stoppers. The reagents were tested chemically for metal traces, with negative results. (Reagents stored in soft glass bottles for relatively short times give abnormally high rates of oxidation of thioglycolic acid.) The thiol itself was kept in sealed Pyrex bulbs, a diluted stock solution being kept no longer than 1 month. The great sensitivity of the thiol to oxidation in the presence of minute amounts of heavy metal ions (about 1 gm. atom of copper or manganese in 2 million cc. of solution) makes it imperative to take all possible precautions against contamination.

The metal catalysts were introduced in the form of dilute solutions of the sulfates (ferrous ammonium sulfate in the case of iron). The carbonate buffers were dilute solutions of sodium carbonate. Other buffers were prepared according to the directions of Clark (1928).

Treatment of Experimental Data—The data obtained for the metal-catalyzed oxidation of thioglycolic acid do not fit the usual reaction equations. The rate at which the thiol is oxidized, however, is linear for most of the reaction (from 5 to 50 per cent oxidation at least), so that $(d \text{ moles R—SH})/dt$ is constant during this time.¹ The oxidation rate is, therefore, concisely expressed by a single number, the slope of this line. To avoid fractions, moles of thiol are multiplied by 10^6 ; time is in minutes.

The manometric data are expressed as usual in c.mm. per unit of time, though the unit chosen here is 100 minutes rather than 1 hour. As above, only the linear portion of the oxidation is used to obtain a value. Results are reproducible within ± 0.02 c.mm.; and I have, in fact, found it satisfactory to calibrate new manometer vessels by charging them with a known amount of thiol and tipping in copper after equilibrium is reached in the thermostat. Although the two notations do not give directly comparable numbers, this is irrelevant for most purposes, as complete experiments were performed in both cases.

Results

Temperature and Oxygen Concentration—Fairly complete data on the influence of temperature and oxygen concentration on this oxidation are available (Elliott (1930); see also Elvehjem (1930) on cysteine oxidation); therefore my own similar findings are briefly summarized.

Under similar conditions, the rates of oxidation of 0.014 M thioglycolic acid at 20° and 1.6° are 116 and 52, respectively (rate in moles $\times 10^6$ R—SH oxidized per minute, carbonate buffer at pH 8.7; 10^{-5} M Cu^{++}). Since a decrease in temperature of 18° is accompanied by a decrease of 55 per cent in rate, the change is roughly 3 per cent per degree. Obviously a temperature fluctuation of $\pm 0.1^\circ$ does not affect results.

Fig. 2 illustrates the influence of oxygen concentration and rate of flow upon thiol oxidation (at 20°, other conditions as above). It is interesting that, below optimal concentration and attendant

¹ This constancy, also noted by Elliott (1930), suggests that metal and thiol react at once to give a fixed amount of active catalyst which does not decompose as thiol is lost; or that some accelerating factor accumulates as thiol decreases.

maximal constant rate of oxidation, changes in the concentration of dissolved oxygen markedly affect the oxidation rate, even though diffusion or mixing with the reactants is not a factor. With pure oxygen, 4 liters per hour permits maximal rates. Extrapolations of the three curves, for different oxygen supply, cross the time axis at the same point, indicating a lag of 2 minutes. This can hardly be time required for solution of oxygen to the equilibrium value, for the solution is in equilibrium with the gas before the catalyst is added. More probably during the induction period an active catalytic substance is being formed.

Initial Thiol Concentration—To test for metal catalysis, low

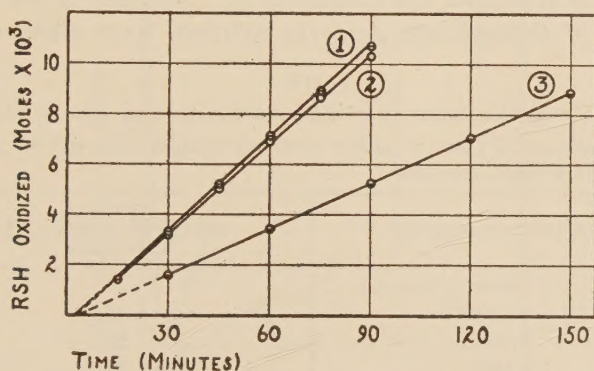


FIG. 2. Influence of oxygen supply upon the rate of oxidation of 0.014 M thioglycolic acid at 20° in carbonate buffer in the presence of cupric ions (9.5×10^{-6} M). Curve 1, oxygen supplied at the rate of 8 liters per hour; Curve 2, oxygen at 4 liters per hour; Curve 3, air at 4 liters per hour.

thiol concentrations are preferable to high because of the greater sensitivity of a dilute system to small absolute amounts of metal ions, and because in more concentrated mixtures metal salts are likely to precipitate with buffer or other radicals. A test of high absolute sensitivity is desirable for the small quantities of the extracts available from tissues. Oxidation rate increases with thiol concentration, but very slightly when the concentration is high enough, presumably, to keep the catalytic ions almost continuously bound.

My results (Table I) confirm the observation of Elliott (1930) that, in the presence of copper ions, the rate of oxidation increases with the initial thiol concentration, more rapidly at low concentra-

tions than at high. The most dilute solution is completely oxidized in 100 minutes, the most concentrated only 27 per cent, so that the former affords a more sensitive test for metals; and in anticipation of later work I have chosen a fairly dilute thiol for my regular procedure.

From the difference in rates for the two lowest initial thiol concentrations recorded in Table I, it may be calculated that a change of 1 per cent in initial concentration leads to a change of only 0.3 per cent in rate. Accordingly, even a 10 per cent error in concentration would affect the rate by only 3 per cent, which is within the limit of accuracy set by other factors.

Effect of Hydrogen Ion Concentration. Influence of pH on the Stability of Dithioglycolic Acid—In solutions more alkaline than

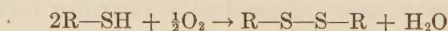
TABLE I

Effect of Initial Concentration of Thioglycolic Acid on Rate of Oxidation

Temperature, 20°; 10^{-5} M cupric ions; carbonate buffer, pH 8.7; oxygen supplied at 4 liters per hour.

Initial thiol concentration	Rate Moles R-SH oxidized $\times 10^6$ Time in min.
M	
0.200	54
0.050	22
0.025	16
0.012	12

pH 9, the dithiol formed becomes unstable and is further oxidized, so that oxidation rates are not fully comparable with those in the more acid range and the total oxygen used exceeds the theoretical requirement for the simple equation



To study the stability of dithioglycolic acid in alkaline solutions, a known amount of the thiol was oxidized without decomposition at pH 6.0, the oxygen was removed by prolonged passage of nitrogen (rendered oxygen-free by forcing it through a porcelain candle in alkaline sodium hydrosulfite), and, without interrupting the nitrogen stream, the solution was brought to pH 8, 9, or 12, in separate tests, by adding oxygen-free alkali solution. Samples were then drawn from the reaction bulb at intervals and titrated

in the usual manner. At pH 12, the solution reduced a steadily increasing amount of iodine; at pH 8 or 9, a small constant blank titration was obtained; so that only at high pH does the dithioglycolic acid progressively decompose to form reducing substances.

In another experiment, thioglycolic acid, in the presence of cupric ions, was oxidized as usual, but at pH 12, and the iodine titrations were continued for 3 hours. The minimum titer was

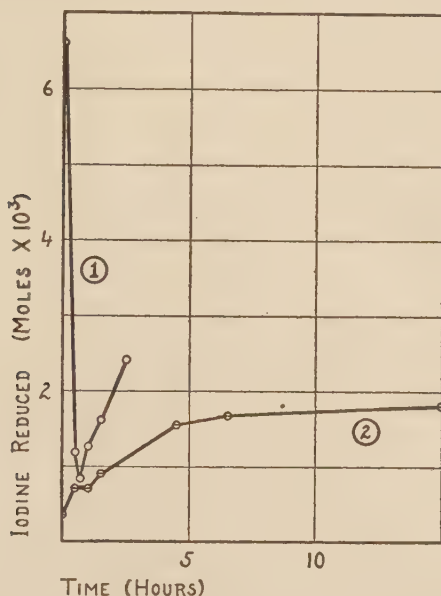


FIG. 3. The instability of dithioglycolic acid at pH 12. Curve 1, 20° 4 liters of oxygen per hour; initial concentration of thioglycolic acid, 0.014 M; 10^{-5} M cupric ions; Curve 2, the same, except that oxygen was replaced with nitrogen.

reached in less than 1 hour, after which it rose (Fig. 3), again showing the instability of the dithiol at this pH (Wieland and Franke, 1928; Dixon and Tunnicliffe, 1923).

Influence of pH on Metal Catalysis of Thiol Oxidation—Since the addition of buffers is essential in any study of the influence of pH, it is sometimes difficult to discriminate between the effect of hydrions *per se* and of the buffer ions. This is particularly true in the present study since, as will be shown, the usual buffers pro-

foundly influence the catalytic activity of all the metal ions, and no one buffer covers a sufficiently extensive pH range. The catalytic rates with copper, however, were identical in such differ-

TABLE II

Influence of pH on Copper Catalysis of Thiol Oxidation (Titrimetric Measurements)

Temperature, 20°; 0.014 M thioglycolic acid; 10^{-5} M cupric ions; oxygen supplied at 4 liters per hour.

Buffer*	pH	Rate Moles R-SH oxidized $\times 10^5$ Time in min.
Carbonate†	3.0	0
Phthalate	5.0	4
“	6.0	16
Orthophosphate	6.0	16
“	7.0	11
“	8.0	11
Borate	8.0	10
“	9.0	13

Buffer	Catalyst, M $\times 10^5$	pH	Rate
Orthophosphate	7 Fe ⁺⁺	6.0	84
Borate	7 “	9.0	21
Carbonate	7 “	8.2‡	43
“	7 “	8.7	60
“	5 Mn ⁺⁺	8.2	74
“	5 “	8.7	92

* With the exception of the carbonate, all buffers were prepared according to the directions of Clark (1928). The final crystallizations of the salts were made with the use of doubly distilled water.

† In this experiment the system can scarcely be properly said to have been buffered. Enough Na₂CO₃ was added to bring the system to pH 3.15. After 90 minutes no oxidation had occurred, and the final pH was 3.20.

‡ To maintain the pH at 8.2 it was found convenient to pass from 7 to 10 per cent of the oxygen through a saturated bicarbonate solution at 20°. Otherwise, the pH of a carbonate-buffered system rapidly rises under these conditions from an initial value of 8.2 to 8.7, where it remains constant.

ent buffers as orthophosphate and phthalate at pH 6, and agreed within 10 per cent at pH 8 in orthophosphate and borate, so that it may reasonably be assumed that differences evident through the pH range are largely dependent upon the changing hydrion

concentration. The rate of oxidation of thioglycolic acid in the presence of copper was determined at pH values of 3, 5, 6, 7, 8, and 9. The rate increases from negligible values at pH 3 to a maximum at 6, is diminished at pH 7 and 8, and at 9 rises toward the maximum at 6 (Table II). The complication introduced by specific buffer effects in manganous and ferrous ion catalysis is indicated in the lower section of Table II. In carbonate, rates for each ion increase with alkalinity between pH 8 and 9.

Buffers—The influence of some common buffers (especially those containing ions encountered in tissue preparations) on the various metal catalyses was rather fully examined. Comparisons of two were, of course, performed at the same pH, and most experiments were carried out at pH 8 to 8.6. In certain cases the pH tended to drift toward the alkaline side during a run, giving a positive error in the individual sample but not invalidating the general relations.

Carbonate—The changes produced by various phosphates on metal catalyses are referred in the following discussion to the metal activities in carbonate as a standard. It is difficult to justify fully the choice of carbonate buffer as a reference point, since the carbonate ion may exert a specific effect, just as do the phosphates. However, from data on hand (Tables IV, V, *a*, VI), I believe that catalytically the carbonate ions are relatively inert. True, the lower activity of copper in carbonate than in pyrophosphate (Table III) may represent depression by the former rather than acceleration by the latter; but, since the effect of pyrophosphate varies with its concentration and that of carbonate does not, it seems reasonable to attribute the specific action to the pyrophosphate. Carbonate is in general preferable for the study of tissue catalysts; the disadvantages of phosphate are indicated in more detail in the next section, and borate, while permitting the catalytic action of all these metals (Warburg, 1927), is difficult to free of its metal impurities, and also forms a variety of organic complexes which might disturb results with tissue extracts. The buffer action of carbonate is not very strong at pH 8, but in experiments in which it is used the pH rarely altered over 0.1 unit during the run, an inconsiderable change.

The interactions of metal ions in carbonate buffer are strikingly different from those in phosphate. Copper and iron, and man-

ganese and iron give simple additive effects when present together; while copper and manganese together exhibit a tremendously increased action.² Thus, a rate of thiol oxidation of unity in the presence of 1 unit of copper is more than doubled by the addition of 0.1 unit of manganese, itself only one-third as active as copper (see Table VII, *a*). Since small amounts of manganese added to larger quantities of copper produce a greater acceleration than the converse system, it seems probable that the manganese acts as accelerator to the copper.

Phosphates—Since Warburg (1927) early found that pyrophosphate markedly affected the oxidation of cysteine under metal catalysis and much other work indicates an important rôle of phosphates in biologic oxidations, the sodium salts of ortho-, meta-, pyro-, and certain organic phosphates were studied in relation to the catalytic influence of copper, iron, and manganese on the thiol oxidation. The catalytic action of iron (assuming activity in carbonate buffer as standard) is decreased about 50 per cent by orthophosphate.³ This effect is but little greater when the molar ratio of phosphate to ferrous ion is 10,000 than when it is 100 (see Table III, second column). Results with metaphosphate were less regular but also indicate a depression of about 40 per cent in the catalytic activity of iron when the salt is present in molar ratios of from 10 to 10,000. Most inactivating is pyrophosphate, which, even when present in the low ratio of 4 moles to 1 of iron, gives a 60 per cent lowering, and in a ratio of 5000:1 suppresses 90 per cent of the iron catalysis.

Manganese is still more sensitive to the various phosphate ions, all of which depress its action progressively with increasing concentration. Thus for orthophosphate the activity loss is 25 per cent at a molar ratio of 100 and 91 per cent at 10,000; for metaphosphate, 30 per cent at 4, 92 per cent at 160, 96 to 99 per cent at 3000.

² Warburg (1931) reports a somewhat similar interaction between iron and manganese for the oxidation of cysteine in alkaline borate solution with cyanide present.

³ Orthophosphate contains metal impurities that cannot be removed by repeated crystallization but which do settle from a stock solution after a week or more as insoluble complexes. Lower rates are therefore obtained in "aged" solutions. I have used such solutions since the situation became clear.

TABLE III

Influence of Phosphates on Metal Catalysis of Thiol Oxidation (Titrimetric Measurements)

Temperature, 20°; 0.014 M thioglycolic acid; sodium carbonate stock solution used to adjust pH; initial pH 8.2 in all cases; oxygen supplied at 4 liters per hour. Rate calculated in terms of (moles of R—SH oxidized $\times 10^6$)/(time in minutes).

	Molar ratio, phosphate to metal ion	Rate			Final pH
		Fe ⁺⁺ (4×10^{-6} M)	Mn ⁺⁺ (5×10^{-6} M)	Cu ⁺⁺ (5×10^{-6} M)	
Orthophosphate	0:1*	(43)†	74	38	8.2
	100:1	21	55		8.3
	1,000:1	20	34		8.3
	2,500:1	20	21		8.2
	5,000:1	15	13		8.2
	10,000:1	17	7	48	8.2
	20,000:1		7		8.2
Metaphosphate	0:1*	(43)†	74	38	8.2
	100:1	22	67	112	8.6
	500:1		27		8.7
	1,000:1	14	10	126	8.7
	10,000:1		4	182	8.6
Pyrophosphate	0:1*	(43)†	74	38	8.2
	4:1	15	32		8.8
	8:1		20		8.8
	16:1	11	10	66	8.8
	160:1	13	6	60	8.6
	1,330:1			69	8.5
	2,660:1		1	70	8.4
	3,530:1		3		8.4
	5,320:1	3		78	8.4
	21,250:1			112	8.3
	42,500:1	3	3	113	8.2

* Refers to the rates obtained in a carbonate buffer.

† The rate characteristic of this concentration of iron ions was not determined at pH 8.2. However, at double this concentration ferrous ion is inhibited to the extent of 42 per cent in a phosphate buffer as compared to carbonate.

The activity of copper, on the contrary, is increased by these buffers. Pyrophosphate in a molar ratio of 16 gives a 65 per cent increase in the catalytic effect; of 2500, 80 per cent; and of 40,000,

190 per cent.⁴ Orthophosphate acts in a similar, though less powerful, manner; and metaphosphate may be even more effective.⁵ Experimental values are summarized in Table III.

It may well be emphasized that these results cannot be transferred freely from one substrate to another. For example, pyrophosphate does not augment but depresses the action of copper in catalyzing the oxidation of carbohydrates (Krebs, 1927).

Sodium Glycerophosphate—Sodium glycerophosphate (also, in a few experiments, hexosemonophosphate) is inactive or slightly accelerating as regards iron and manganese catalyses, although

TABLE IV

Influence of Sodium Glycerophosphate on Thiol Oxidation by Metals

Temperature, 20°; 0.014 M thioglycolic acid; oxygen supplied at 4 liters per hour. Rate calculated in terms of (moles of R—SH oxidized $\times 10^6$)/(time in minutes). Catalysts, 5×10^{-6} M Cu^{++} , 8×10^{-5} M Fe^{++} , 5×10^{-6} M Mn^{++} ions.

Buffer	pH	Addition	Catalyst	Rate
Carbonate	8.2		Cu^{++}	38
"	8.2		Fe^{++}	43
"	8.2		Mn^{++}	74
"	8.2	0.02 M glycerophosphate		3
"	8.2	0.02 " "	Cu^{++}	46
"	8.2	0.02 " "	Fe^{++}	47
"	8.2	0.02 " "	Mn^{++}	84
"	8.2	0.02 " orthophosphate	Fe^{++}	25
"	8.2	0.02 " "	Mn^{++}	14
Orthophosphate (0.02 M)	8.1	0.02 " glycerol	Fe^{++}	20

the glycerol alone, as well as the orthophosphate, is inhibitory. Aside from the chemical significance of such findings, they can hardly fail of considerable biologic significance, since the normal metabolic traffic in tissues involves continuous formation and loss of ortho-, pyro-, and several organic phosphates, with a probable consequent shift in catalytic activity of metal ions.

Table IV illustrates the difference in action of sodium glycerophosphate.

⁴ Compare with similar findings on cysteine oxidation (Elvehjem, 1930).

⁵ Elliott (1930) reports an inhibitory effect of orthophosphate on copper catalysis.

phosphate and sodium orthophosphate on iron and manganese catalyses.

Effect of Metal Ion Concentration—The marked increase of catalytic effect with increasing copper concentration, in pyrophosphate buffer is shown in Fig. 4. Data for other ions and buffers as well are given in Tables V, V, *a*, and VI. The catalytic activity of copper in pyrophosphate buffer, as measured by the rate of thiol oxidation, is not a linear function of the metal concentration, but is roughly proportional to its square.

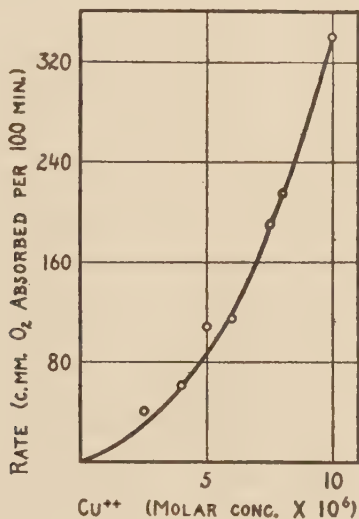


FIG. 4. Effect of cupric ion concentration on the rate of oxidation of thioglycolic acid in pyrophosphate buffer at 20°.

Mutual Interaction of Metal Ions—It is significant that iron and manganese, although almost inactive individually in a pyrophosphate buffer, are still able to influence the action of copper. Manganese depresses the catalytic activity of copper by 20 to 30 per cent, while iron increases it by 10 to 20 per cent. When both ions are added to copper, the inhibitory effect prevails. Again the nature of the substrate appears important, since, for cysteine oxidation, Warburg (1927) found no interaction between copper and manganese. In carbonate, manganese and copper together have an increased catalytic activity.

TABLE V

Effect of Metal Ion Concentration on Rate of Oxygen Absorption by Thiol in Pyrophosphate (Manometric Measurements)

Temperature, 20°; 0.05 M sodium pyrophosphate buffer; pure oxygen atmosphere; original thiol concentration, 0.014 M.

Cu ⁺⁺		Mn ⁺⁺		Fe ⁺⁺	
M × 10 ⁵	O ₂ absorbed per 100 min.	M × 10 ⁵	O ₂ absorbed per 100 min.	M × 10 ⁵	O ₂ absorbed per 100 min.
	<i>c.mm.</i>		<i>c.mm.</i>		<i>c.mm.</i>
2.5	40	2	3	10	2
4.0	61				
5.0	108	10	3		
6.0	115			20	3
7.5	190	20	3.5		
8.0	215			50	4
10.0	340	100	1		

TABLE V, a

Effect of Buffers and Metal Concentration on Rate of Thiol Oxidation (Titrimetric Measurements)

Temperature, 20°; initial thiol concentration, 0.014 M; oxygen supplied at 4 liters per hour. Rate calculated in terms of (moles of R—SH oxidized × 10⁶)/(time in minutes).

Buffer, 0.002 M	Catalyst, M × 10 ⁵	pH	Rate
Phthalate	5 Cu ⁺⁺	5.0	11
"	10 "	5.0	28
"	5 "	6.0	80
"	10 "	6.0	160
Orthophosphate	5 "	6.0	77
"	10 "	6.0	174
"	5 "	8.0	48
"	10 "	8.0	104
Borate	5 "	8.0	45
"	10 "	8.0	99
Orthophosphate	7 Fe ⁺⁺	6.0	84
"	14 "	6.0	190
Borate	7 "	9.0	21
"	14 "	9.0	42

Tables VII and VII,a record the experimental results obtained by the manometric and titrimetric methods respectively. For convenient comparison, calculated additive rates from Tables V and VI are included.

Effect of Dithiol—Harrison (1927), following the lead of Dixon and Tunncliffe (1923), has demonstrated an autocatalytic oxidation of thiols in the absence of heavy metals. The oxidation follows a sigmoid curve and is definitely accelerated, in the early stages, by addition of dithiol. Gerwe (1931) likewise has obtained autooxidation of cysteine under presumably metal-free conditions (but see Elvehjem, 1930). The catalytic action of the dithiol is, however, greatly enhanced in the presence of metals, so that I

TABLE VI

Effect of Metal Concentration on Rate of Thiol Oxidation in Carbonate Buffer (Titrimetric Measurements)

Temperature, 20°; 0.014 M thioglycolic acid; carbonate buffer; oxygen supplied at 4 liters per hour. Rate calculated in terms of (moles of R—SH oxidized $\times 10^6$)/(time in minutes).

Catalyst, M $\times 10^6$	pH	Mean rate	No. of experiments in mean	Maximum variation from mean
				<i>per cent</i>
5 Cu ⁺⁺	8.2	38	3	8
80 Fe ⁺⁺	8.2	43	3	7
5 Mn ⁺⁺	8.2	74	2	2
5 Cu ⁺⁺	8.7	49	10+	9
10 "	8.7	122	8	5+
20 "	8.7	266	1	
40 Fe ⁺⁺	8.7	22	7	8+*
80 "	8.7	60	3	10
0.3 Mn ⁺⁺	8.7	11	10+	8
0.6 "	8.7	21		
1.2 "	8.7	28		
5.0 "	8.7	92		

* Does not include three results that deviate greatly from the mean.

have been able to note distinct effects with relatively small (compared to Harrison's experiments) proportions of dithiol added to the thioglycolic acid.

As could now be anticipated, the dithiol influence varies according to the metal added, the buffer, etc., but under all conditions tried it is in the positive direction. 1 part of dithiol added to 10 of thiol, for example, leads to the following increases in rate of oxidation; for copper in orthophosphate 30 per cent, copper in carbonate 56 per cent, iron in orthophosphate 28 per cent, iron in

carbonate 19 per cent. It will be noted that the increased activity induced by the dithiol for each metal ion is greatest in the buffer in which the initial activity is least. Along the same line, the

TABLE VII

Effect of Iron and Manganese Ions on Copper Catalysis in Pyrophosphate (Manometric Measurements)

The experimental constants were the same as for Table V.

Catalysts ($M \times 10^6$)			O ₂ absorbed per 100 min.	
Cu ⁺⁺	Mn ⁺⁺	Fe ⁺⁺	Calculated, additive	Observed, combined
			<i>c. mm.</i>	<i>c. mm.</i>
7.5	10.0		193	140
10.0	10.0		343	280
7.5	20.0		194	140
8.0	100.0		216	142
10.0	20.0		343	260
10.0		10.0	342	410
10.0		20.0	344	400
8.0		50.0	219	252
7.5		10.0	192	210
7.5		20.0	194	220
10.0	2.0	20.0	347	310
8.0	2.0	50.0	223	174

TABLE VII, a

Mutual Augmentation of Catalytic Activities of Copper and Manganese in Carbonate Buffer (Titrimetric Measurements)

The experimental constants were the same as for Table VI.

Catalysts ($M \times 10^6$)		Rate	
Cu ⁺⁺	Mn ⁺⁺	Calculated, additive	Observed, combined
5.0	5.0	141	202
5.0	1.2	77	155
5.0	0.6	70	109
5.0	0.3	60	76

activity of manganese, largely lost in orthophosphate, is mostly restored by the addition of dithiol. In carbonate, there is a much smaller percentage increase, though the absolute effect is about the same as in phosphate.

TABLE VIII

Effect of Dithiol on Rate of Oxidation of Thiol by Metals in Orthophosphate and Carbonate Buffers (Titrimetric Measurements)

Temperature, 20°; 0.014 M thioglycolic acid; oxygen flow, 4 liters per hour. Rate = (moles of R—SH oxidized $\times 10^5$)/(time in minutes).

Buffer	Dithiol concentration M $\times 10^4$	Rate			
		No metal added	Cu ⁺⁺ (5×10^{-5} M)	Fe ⁺⁺ (8×10^{-5} M)	Mn ⁺⁺ (5×10^{-5} M)
Orthophosphate, pH 8.1	0	1	56	25	8
	14		63	32	
	35		71	41	17
	70		90	60	32
	140	9			
Carbonate, pH 8.2	0	1	38	43	74
	14		64	55	
	28			64	
	42			71	
	70		73	99	95
	140	9			

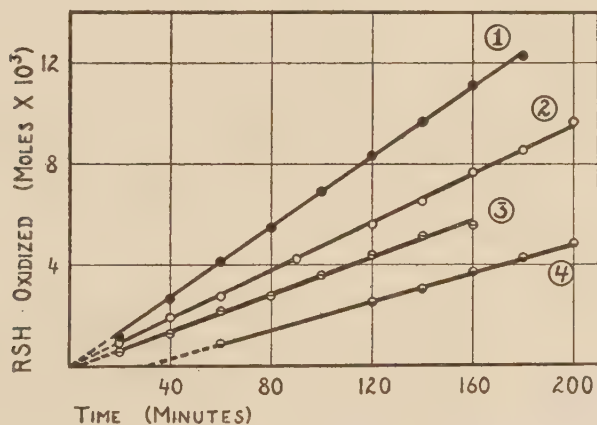


FIG. 5. Influence of addition of dithioglycolic acid to a thioglycolic acid oxidation system in orthophosphate buffer. Temperature, 20°; iron catalyst (8×10^{-5} M); initial concentration of thioglycolic acid, 0.014 M. Ratio of dithiol to thiol, Curve 1, 1:2; Curve 2, 1:4; Curve 3, 1:0; Curve 4 no dithiol.

When the amount of dithiol added is varied, further differences emerge. Thus, for copper, the accelerating effect of dithiol is about proportional to its concentration in orthophosphate buffer, but in carbonate small amounts are relatively more effective. In the case of iron, the dithiol effect varies with its concentration in both buffers. Table VIII illustrates the marked acceleratory influence of dithioglycolic acid on the metal catalysis of thioglycolic acid oxidation.

In Fig. 5 the influence of the addition of various ratios of dithiol to an iron-catalyzed orthophosphate thiol oxidation system is graphically illustrated. The slopes of the curves are, of course, proportional to the respective rates of oxidation.

The degree of change with inhibitor and accelerator substances will depend on the relative concentrations of thiol and active substance, the relative stabilities of the metal complexes formed with each, and the relative catalytic effectiveness of the complexes formed. When the first two relations are favorable and the third positive, that is, when the new complex is more effective as a catalyst than that with the thiol, then acceleration results, as with dithiol. When the new complex is less effective or inactive, inhibition occurs as in the classical case with cyanide.

SUMMARY

1. The catalyzed oxidation of thioglycolic acid by oxygen in the presence of metal ions has been studied by the manometric measurement of oxygen used and by iodine titration of the thiol remaining.

2. Results obtained as temperature and oxygen content are varied are summarized.

3. The influences of hydron concentration and specific buffer effects were examined, especially on copper catalysis. With this ion, the rate increases from 0 at pH 3 to a maximum at 6, falls again to 8, and rises sharply at 9. At greater alkalinity the disulfide undergoes oxidative decomposition.

4. Carbonate buffer has little specific influence on the catalysts studied. In this, combinations of iron with copper or manganese show an activity equal to their summed separate actions. Copper and manganese, however, are tremendously more active together than apart.

5. Ortho-, meta-, and pyrophosphates exert marked influences on iron, manganese, and copper catalyses. The three inhibit iron with increasing power in the order given, small amounts of buffer being almost as effective as much larger quantities. Manganese is even more sensitive, the various phosphates inhibiting progressively more with increasing concentration. Copper, on the other hand, is accelerated by the phosphates, activity increasing roughly as the square of the metal ion concentration.

6. Buffers made from glycerophosphate and hexosephosphate do not affect catalyses by iron and manganese.

7. Copper catalysis in phosphate buffers is decreased by the simultaneous presence of manganese, increased by iron.

8. Dithiol, present at the start, hastens thiol oxidation by all metals and in all buffers used. Special quantitative relations are described in the text.

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